FLORAL MANIPULATION IN MANGOS

Tom Davenport
Department of Horticulture
University of Florida

My research on mango flowering began about five years ago. By that time, smudging, the traditional Philippine use of smoke to promote flowering, had given way to the more convenient and efficacious use of ethephon (a compound that generates ethylene in plants) and potassium nitrate sprays. Not only were mango trees in the Philippines stimulated to flower out of season with these treatments, but irregularly-bearing trees could be stimulated to bear in most years. The connection between smoke (which contains ethylene), ethylene generated from ethephon, and flowering response led to the hypothesis that ethylene was the "hormone" which induced trees to flower.

Based on what we knew at the time, ethylene was a potential factor in flowering. In support of the hypothesis, we had observed epinasty, the temporary turning-under of leaves, occurring in leaves of flowering branches. Those involved in ethylene physiology recognize epinasty as one symptom of ethylene exposure, either endogenously produced or exogenously applied as a gas. Therefore, early in our experiments we measured ethylene production in buds, leaves, and developing panicles. The results of a number of experiments led us to the conclusion that enhanced ethylene production does not seem to be involved in mango flowering. We found that floral buds which should have been producing ethylene were not producing significantly more than plant parts at other stages of growth. The levels of ethylene observed in flowers were basically the same as background levels. We applied ethylene in the form of ethephon, causing the tissues to produce copious amounts of ethylene. It resulted in no stimulation of flowering. Moreover, potassium nitrate did not increase ethylene levels or stimulate flowering in either 'Tommy Atkins' or 'Keitt' trees.

Potassium nitrate (KNO_3) came into general use in the Philippines in the 1970s. It too was speculated to stimulate flowering through a wound-ethylene response. It now is widely used in Mexico as well. Although responses may occur at concentrations ranging from 1 to 8 percent, Mexican growers generally use 4 percent KNO_3 or 2 percent ammonium nitrate. Leaf tip burn also occurs in dry areas at these concentrations. The flowering response is cultivar-specific. 'Haden', 'Irwin', 'Carabao', and 'Manila', for example, respond well. Polyembryonic cultivars appear to respond most effectively. Response in others, such as 'Tommy Atkins', is more difficult to obtain.

The first dates in which they are able to get an efficacious response in responsive cultivars is in late October in the southernmost area of Chiapas, Mexico. Efficacy decreases, in terms of prolonging the date of first flowering response and increasing the amount of chemical necessary to obtain a response, in trees planted at latitudes further north. Growers in the state of Colima (mid-Mexico) stimulate early flowering by starting sprays in mid to late November. Trees growing in the area of Vera Cruz begin to respond slightly later in the year but lose the ability to respond altogether in areas north of 23° latitude. I have been told that even concentrations sufficiently high to cause substantial leaf burn (10 percent or more) are apparently not effective. Trees located in both Sinaloa (25° latitude, dry climate) and Homestead, Florida (25° latitude, dry climate) do not respond. This is also true for other higher-latitude areas such as in northern India, Australia, South Africa, and Israel.

Because only sections of trees flower in response to sprays, applications are made every two weeks. Generally, other sections of the trees flower with each application. If it occurs, the flowering response is virtually immediate, with buds swelling within two weeks after application. Full flowering occurs within one month.

One must be careful in interpreting such information. Many have found that if KNO_3 is applied too early in the season, they obtain a vegetative instead of a flowering growth response. The same is true for spring or summer applications. It is likely that KNO_3 is not inducing flowering directly, but is stimulating initiation of growth. If conditions are present to induce flowering, then growth will be reproductive. If, on the other hand, conditions are more favorable for vegetative growth then, that will be the response. This point is further discussed below.

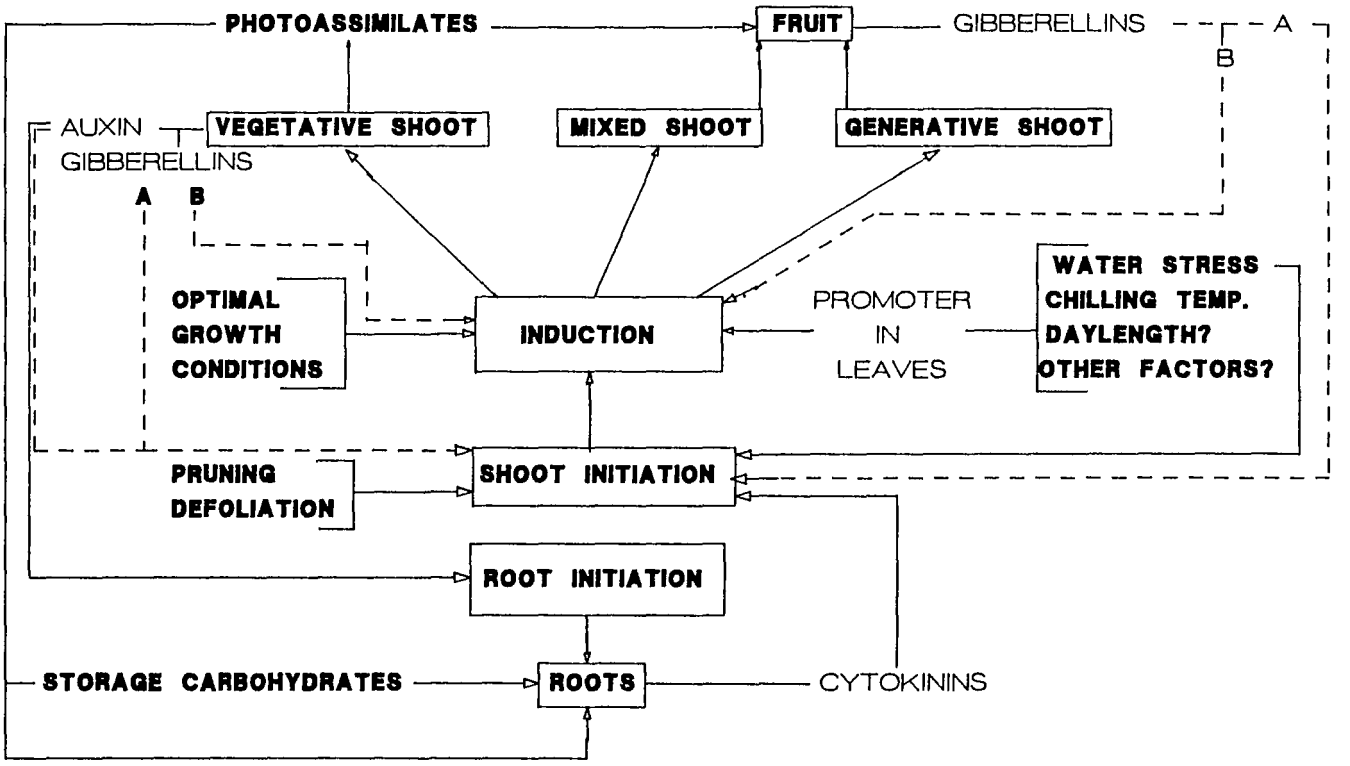

Figure 1. Conceptual model of mango flowering and vegetative growth.

In our research, we needed to produce large numbers of uniform, small plants for use in growth chamber studies. We could not use seedlings because of their juvenility characteristics; juvenile plants would not flower even when exposed to floral-inductive conditions. Experimental plants are produced by air layering, using an auxin, naphthaleneacetic acid (NAA), applied in lanolin to help stimulate root production in the air layer. Rooted air layers are planted in one-gallon pots for use in greenhouse and controlled environment studies. They can be manipulated by pruning or defoliation to manage initiation of new shoots or control leaf age. Mostly, we manipulate them by putting them into defined environmental conditions where we can investigate the effects of temperature, daylength, water stress, etc.

We have developed a conceptual model of flowering and vegetative growth (Figure 1). We are certain about some concepts which are incorporated into the model. Other concepts (such as the role of phytohormones, etc.), are hypotheses based on supportive literature on other plants. The model is thus in one sense a fairy tale, because we have not proved that all its relationships are true; however, it is a useful framework around which we can plan, conduct experiments, and test various hypotheses. So far, everything we have observed in the field seems to fit the model. The model is based on events occurring to individual buds and the forces impacting on those buds which direct its growth. In mango, clusters of stems tend to flush at the same time, although the entire tree may not do so. Upon close observation, one will generally find that these stems are ultimately connected at some common branch point. An astute observer will note that individual buds on mature mango trees rarely grow during the year. They flush only two or three times per year. One can clearly see the history of those flushes recorded in the branches.

There are two distinct switches that have to be turned on for flowering to occur. First, the shoot itself must be initiated to grow; something must

cause the bud to go from a resting state to a growing state. I call this initiation. Once it begins to grow, the second switch has to be turned one way or the other to determine what kind of growth will occur: vegetative (producing leaves) or generative (producing a panicle). Sometimes, a confused mixture of the two is produced, which we call a mixed shoot.

If shoot initiation occurs when optimal growth conditions (warm, humid weather) prevail, it will develop into a vegetative shoot. The photoassimilates which the resulting leaves produce provide food for development of roots and other vital plant organs including fruit when available. They are either used immediately or stored in locations throughout the tree to be used at times when demand for carbon resources is greater than the current photosynthetic supply.

Vegetative shoots and fruit are also well known to be sources of two classes of plant hormones: auxins and gibberellins. These phytohormones may be involved in an internal cycle which regulates shoot initiation. For example, auxin is actively transported to roots from sites of production in shoots. Auxins are well known to stimulate root growth. This flush of root activity may either be a transient effect, or roots may grow somewhat continuously. Preliminary results in our lab and extensive research reported on other species indicate that the former may be the case, but results of others support the latter possibility. Regardless, shoots are rich in auxins as they develop; auxins are transported specifically downward from the shoot to roots, and as leaves age (the apical buds having gone back into the non-growing, rest stage) we assume (based on supportive research on other plants) that their auxin production declines. Thus, pulses of auxins may stimulate root initiation after vegetative flushing. The roots that develop from growth stimulation are known to be rich sources of cytokinins, which are major factors in stimulating shoot initiation.

We also know, however, that auxin is an inhibitor of shoot initiation. Auxin enforces apical dominance by preventing buds beneath the apex of stems from shooting. We envision a balance of shoot-produced auxin, diminishing as leaves age, and cytokinins in buds gradually increasing as they are transported upwards to buds and leaves through the xylem transpiration stream.

The initiation switch may be, therefore, dependent upon a balance of the two phytohormones rather than the absolute concentration of either one. High auxin levels, compared to cytokinin levels, may inhibit shoot initiation, and high cytokinin levels, compared to auxin levels, may stimulate shoot initiation. During a rest period, auxin is possibly decreasing, cytokinins are increasing, and at some point, the bud's initiation switch is triggered, stimulating it to grow. This conceptual model predicts that we should see initiation of buds in response to increasing cytokinins and decreasing auxins levels, and that in an opposing root cycle we would get the opposite conditions resulting in flushing of roots. The literature on apples and citrus supports this type of alternating flushing behavior. Our preliminary experiments, thus far, support these hypotheses. Nobody, however, has done the experimental work with mango, because it is difficult. You have to separate growing sections of the tree from other sections.

There is evidence that cytokinins have the effects that our model predicts. We have applied a synthetic cytokinin, such as 100 ppm thidiazuron, to resting buds. We obtained tremendous shoot initiation and proliferation in several experiments. If applied during an inductive period, i.e., the wintertime, we got proliferation of inflorescences; if applied during the summertime under non-inductive conditions, we got either normal shooting or a proliferation of shooting.

When buds begin to grow they are apparently influenced by ambient environmental conditions which determine the form of newly initiated growth. The floral-inductive condition assumes that a promoter is present in leaves. We and others have demonstrated that leaf removal prevents flowering of new shoots. During an inductive period (cool, winter nights), we girdled branches (to isolate them from the rest of the tree) and deblossomed the same branches (to stimulate new growth), and we defoliated some of those branches on day zero (when we deblossomed and girdled) and did the same to other branches on days two, five, and eight. We confirmed that leaves were required as sensory organs to measure the inductive conditions. All growth resulting from the treatment at days zero and two was purely vegetative. There was an increase in generative shoots following the day-five treatment, with a further increase after the day-eight treatment. Other experiments along these lines showed that with no defoliation at all, 100 percent of the new shoots were generative. We are, thus, fairly confident that leaves are the sensory organ, and the florigenic promoter is a

labile compound that does not stay around for long. At some point from zero to 14 days after stimulating new growth by pinching off the stem apex, the florigenic promoter disappeared. As the time interval between defoliation and emergence of new buds got closer, the influence of leaves retained for longer periods became stronger. There seems to be about a one-week period required for the florigenic promoter to degrade to a point where it is no longer stimulatory.

In another experiment, branches were deblossomed (to stimulate new growth) and defoliated (to remove the florigenic promoter) on day zero, but each branch was girdled, thus isolating it, on day 0, 5, 10, or 15, to see if the putative florigenic promoter is available from other branches. Another set was left not girdled. Even if girdled on day 15, we saw only vegetative growth result. The non-girdled treatment, however, resulted in a reduction in the number of vegetative shoots and an increase in the number of flower-producing shoots. Those shoots were composed mostly of an atypical shoot type which started out purely vegetative but reverted to inflorescence formation in the latter half of shoot development. These were termed transition shoots, in contrast to mixed shoots which form both leaves and inflorescences in the same nodes at the same time. We have been able to duplicate formation of transition shoots in growth chambers by transferring plants from warm temperature to chilling temperature during early bud development. These results indicated that the florigenic component may be moving, possibly in the phloem, but arriving late from other branches to supply buds that were initially lacking a florigenic promoter due to defoliation.

Environmental conditions such as water stress, chilling temperatures, and possibly daylength have been suggested to provide the conditions necessary to induce flowering of mango. We have examined water stress (lack of water) in detail but have found no link of flowering to water relations. We have found the same lack of correlation of flowering with daylength. Chilling temperature, on the other hand, definitely has an impact. The threshold temperature to induce flowering of 'Tommy Atkins' appears to be about 65°F. Chilling temperatures need only to occur at night. Day temperatures are not so critical. Other cultivars likely have different thresholds of induction. At present, we feel that chilling temperature stimulates production of the putative florigenic promoter. It is, thus, reproducibly controllable with environmentally-controlled growth chambers. We are able to stimulate flowering of small containerized plants, propagated by air layering, at any time of the year.

We can also control what we perceive to be a flowering inhibitor (or inhibitors), which appears to occur in leaves as well. The presence and strength of that inhibitor seems to be influenced by the age of those leaves. Apparently, the older the leaf, the less impact the inhibitor has. For example, plants with leaves of different ages were placed in an environmentally-controlled growth chamber and stimulated to grow by pruning. Plants with older leaves flowered, whereas plants with younger leaves grew vegetative shoots. We are investigating the possibility that this inhibitor is a gibberellin, a large class of phytohormones exhibiting a variety of influences on plants from stem elongation to inhibition of growth and flowering. We have applied different levels of GA_3 to branches of both field and greenhouse plants and have found that it inhibited initiation of bud growth. The length of time in which initiation was inhibited was concentration-dependent, but panicles formed when initiation occurred regardless of concentration. Thus, it appears that a gibberellin closely related to GA_3 is involved in inhibition of initiation but not to inhibition of the induction switch. We speculate that there is another gibberellin which acts as an inhibitor of the induction switch. This suggestion is supported by the flower-promoting effects of gibberellin-synthesis inhibitors such as paclobutrazol. Fruit as well as vegetative shoots may produce these inhibitors based on the observed inhibitory effects of their presence on the tree.

Whether or not an initiated bud will be induced to vegetative or generative growth may not depend on the absolute amounts of promoter or inhibitor present in buds, but on the relative balance of the two. This theory may explain the observation that vegetative growth results if young, mature leaves are present on the stems subjected to marginally inductive conditions (high inhibitor, lower promoter) and that generative growth results when the night temperatures are chilling (45-60°F) even in the presence of relatively young leaves (high inhibitor, higher promoter). Similarly, when inductive temperatures are marginal, plants with old leaves flower (low inhibitor, higher promoter), or if plants with old leaves are placed in non-inductive conditions, then they grow vegetatively (low inhibitor, lower promoter). Our research has led us to the conclusion that the

inductive switch is determined at the time of bud initiation, not before.

Flowering and vegetative flushes generally occur in sections of mango trees grown in the tropics, with different sections flushing at varying times. Trees in subtropical areas, which usually receive extended periods of winter chilling night temperatures, tend to produce synchronous flowering flushes, i.e., occurring throughout the tree at once. Trees on Oahu appear to have experienced long periods of cool nights this year. If winter temperatures are warm, then flowering becomes asynchronous similar to the tropical situation. To explain this phenomenon, I suggest that the tree be viewed as a community of organisms instead of one. Each is complete with roots, branches, and canopy. Each sector (organism) is on its own agenda of shoot flushes and root growth. Our experiments have shown that dyes which were applied to roots migrate up trees in the xylem stream to specific branches which are aligned with those roots. Little lateral movement of the dye occurred. The connection of roots to shoots follow their alignment as governed by the architecture of the tree. In order to profitably control flowering, we must create synchrony of growth. This can be achieved by pruning.

Synchronous growth can be initiated by lightly pruning entire trees. Ideally, it would be preferable to supply the flowering promoter at the time growth occurs and hopefully stimulate flowering at any desired time of the year. Unfortunately, no one has identified this putative promoter, much less put it in a bottle. Another way we can manipulate flowering is by manipulating the inhibitor. If, after the post-pruning flush has hardened off, we can stimulate trees to initiate growth with KNO_3, then the timing of that growth can not only be controlled, but made to occur synchronously throughout the tree instead of in patches as is commonly observed when using KNO_3 without synchronization. Trees should be sprayed after sufficient time has elapsed to reduce the level of inhibitor generated from the synchronized flush of leaves and at a time when the inductive conditions of cool temperatures are present to stimulate production of enough promoter to overcome the level of inhibitor.

How can we manipulate the inhibitor? Paclobutrazol is a gibberellin synthesis inhibitor which, when used appropriately, stimulates mango flowering. We have used this fact to connect our putative inhibitor with gibberellins. Application of paclobutrazol in conjunction with KNO_3 can stimulate early synchronized flowering during marginally- or non-inductive conditions when you would never normally see flowering. We believe this is the strategy being used on 'Irwin', 'Parvin', and 'Keitt' in Puerto Rico. They have reported summer flowering of 'Irwin' trees. 'Tommy Atkins' is a different story, because it is recalcitrant in its growth response to KNO_3, but it does respond to paclobutrazol by flowering. We are currently investigating use of cytokinin to stimulate floral initiation in the presence of paclobutrazol.

There are problems with use of paclobutrazol. Because it inhibits the gibberellin syntheses pathway, levels of the gibberellin which is responsible for internode elongation, possibly GA_1, are reduced. Although fruit set and yield may be increased, the product produces a compressed panicle which does not dry out very well and can develop powdery mildew or anthracnose even after a light dew.

Another problem is that when paclobutrazol is applied to soil in excess, under certain conditions, subsequent growth and normal development can be severely disrupted. There is a growing amount of literature on the use of paclobutrazol to get early and more uniform flowering in mangos. No response was observed in seven or eight months after applying paclobutrazol to trees in Homestead. The trees then went through a freeze, our irrigation system failed, and major scaffolding branches were killed. The trees were severely pruned to remove dead wood. The ensuing growth lacked normal node elongation. Trees having only 1 gram of active ingredient applied are still severely stunted after over six years. We investigated the possibility that pruning of the major branches following application was the cause of the undesirable stunting of growth. We applied paclobutrazol, in the same concentration, to trees and waited three years before severely pruning. There was no response to the product until after the trees were pruned. The resulting growth was as severely stunted as before. We believe that this material is chromatographing itself up through the xylem of the tree. It is apparently concentrating itself in main trunks and slowly metering itself out to the branches. When main branches are cut, forcing buds to grow in the area of high paclobutrazol concentration, then you see this strong effect. As long as you do not prune the tree, there appears to be no problem and a many-times limited effect. Recommendations used in Thailand of 1.5 to 2 g/tree/yr to stimulate more uniform flowering may eventually result in this

kind of damage if and when they prune those trees for some reason.

Paclobutrazol is persistent in the soil. If a new tree is planted, it will show the same symptoms. Therefore, we have to be careful when recommending use of such a compound. Experiments are being conducted in Central America on 'Tommy Atkins'. They involve applying paclobutrazol sprays at 30 ppm, which is its solubility in water, to get it to the buds at the proper time to facilitate a flowering response.

In summary, the conceptual model presented in this talk appears to be consistent with growth and development patterns taking place in mango trees all over the world. It predicts what will happen under a defined set of circumstances and is being used to develop strategies which result in flowering at any time of the year. A grower in Puerto Rico utilizing concepts suggested by this model is getting flowering as early as September, and even in July in some cultivars. 'Haden' is an amenable cultivar for manipulation with KNO_3, but 'Tommy Atkins' generally does not respond to this treatment. Potassium nitrate itself does not appear to induce flowering. This point can be verified by spraying trees in the summertime without any positive effect. It is more than likely a combination of the age-dependent inhibitor and whether or not sufficient promoter is available in the leaves that determines the fate of initiated buds. In our hands, we can control both the inhibitory and promotive components. We can make a plant grow when we want it to, and we can make it flower or go vegetative when we want to. This is valuable from the scientific standpoint, because it means we can make biochemical and physiological observations to better understand the interrelationships between the florigenic promoter and inhibitor, and at some point we hope to identify and utilize these components.

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Q: Can't flowering be explained simply by the presence of an inhibitor in leaves rather than a promoter to obtain flowering?

A: No. If this were the case, then we would expect an increase instead of a decrease in flowering response when leaves were removed. We have observed that when one leaf located close to the tip is left on a branch which is otherwise defoliated, the bud just above that leaf will produce an inflorescence, whereas all the other buds will be vegetative. Moreover, the observation that flowering is graft-transmissible can only be explained by the presence of a promoter.

Q: Is paclobutrazol approved for use on any food crop in the U.S.?

A: No.

Q: What is the likelihood that it ever will be?

A: None. That's a problem. I work with several growers in Central America. I have talked to the people at ICI, which manufactures paclobutrazol, and at Sumatomo, which manufactures uniconazol, another product which is about 10 times more efficacious than paclobutrazol. Both companies have no current plans to clear them for use on food products. Paclobutrazol is marketed worldwide with the trade name Cultar for use on avocados, mangos, and other crops, but it is not cleared for use in the U.S. We are applying the material as a solution to branches long before any fruit is on the tree. The likelihood of residue in the fruit is virtually nil, but residue studies have to be done to test that.

Q: Is it possible that paclobutrazol might be approved for foliar application?

A: It's possible but not probable, because the cost of registering these compounds is so great. A company must anticipate a large profit to motivate them to invest the millions required to clear a compound for use. I doubt that would occur because the amounts of product we are using are very small, and the demand for the product in the mango industry overall wouldn't be very large.

Q: Might there be a move to examine mangos being imported into the U.S. for paclobutrazol residues?

A: There might. The only place where paclobutrazol is being used on mangos a lot is in Thailand, where ICI sales reps are strongly promoting its use as a soil drench. Australia is starting to use it as well. The advantage to developing a strategy using sprays at soluble concentrations on foliage prior to flowering instead of soil drenches is that the risk of residue in the fruit is substantially reduced. Regarding the potential stunting effect in pruned trees, I have tried to convince my cooperators in Guatemala and Costa Rica to "hat-rack" prune one of their trees to see the response, but they don't want to sacrifice a productive tree. Stunting too, along with the question of residue, is something that has to be examined.

Q: In the case of cold stress; is there a time factor?

A: In our experience in the growth chambers, it requires a week or two. Basically, those buds that initiate growth in the cold condition are induced to flower. The longer the plants are in the inductive condition, the greater opportunity there is for more buds to initiate panicles. In the field, we have seen that a period of several nights of temperatures down in the 60s is sufficient to cause them to flower, but we have no accurate figures on this. Bear in mind that cultivar differences exist, and the age of leaves varies, both of which factors impact plant flowering response. This year, we had a situation where we had relatively low night temperatures in November-December; they went up in January, then in mid-February they went back down to the 40s and 50s. Our day temperatures are generally in the mid-80s. Sections of some trees that happened to grow during that early part of the season flowered with full panicles. Other sections that grew during the period of higher temperatures grew vegetatively, and sections of the tree that are growing now are producing panicles. That fits with what our model would predict. The lower the temperature, the higher the level of promoter you would expect. I am saying this intuitively, from what we have observed in the field. We have not done experiments, and we do not have a means now to identify or measure this promoter. All we have is the plants' response under given conditions.

Q: Does compaction in the inflorescence as a result of paclobutrazol affect fruiting?

A: Paclobutrazol tends to increase fruit set. On the other hand, too much of the compound compacts panicles to the point where risk of early fruit loss due to disease is increased. The photos you saw were of trees treated with a higher concentration than one would want to use in a normal operating situation. The grower mentioned earlier is synchronizing growth of his 'Irwin' trees by lightly pruning them right after harvest to promote a uniform flush. Then he can regulate the age of his leaves and treat with paclobutrazol about two months later. Although he will not disclose the product he is using, the amount, or how it is applied, I feel certain that he is using a low enough concentration of Cultar to lower the inhibitor level without producing substantial compaction of the inflorescences. He then follows quickly with the KNO_3 to stimulate growth. Basically, he is synchronizing his trees so that all the leaves are the same age, he is reducing the level of inhibitor produced by those leaves with paclobutrazol, and then he is stimulating the tree to grow at that point. It is a smart strategy.